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A Novel Gene That Bears a DnaJ Motif Influences Cyanobacterial Cell Division

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Transposon Tn*5*-692 mutagenizes *Synechococcus* sp. strain PCC 7942 efficiently. The predicted product of the gene mutated in the Tn*5*-692-derived cell division mutant FTN2 has an N-terminal DnaJ domain, as have its cyanobacterial and plant orthologs. *Anabaena* sp. strain PCC 7120, when mutated in genes orthologous to *ftn2* and *ftn6*, forms akinete-like cells.

Division in cyanobacteria, ancient phototrophic relatives of chloroplasts, may serve as a model for the study of chloroplast division. However, the genetic basis of cell division has been studied much less in cyanobacteria than in heterotrophic bacteria (5, 6, 33, 37, 45). Conditional *fts* mutants of *Escherichia coli* affected in cell division were identified by screening for the formation of nonseptate filaments at a restrictive temperature (6, 19, 27, 37). FtsZ (2), a tubulin-like GTPase, forms the basis of a cytoskeletal structure that is used by many bacteria for the mechanical constriction of the cell at the division site (34, 38, 43). Although present in vegetative cells of the cyanobacterium *Anabaena* sp. strain PCC 7120 (12, 50), FtsZ was not detected in the nondividing, differentiated cells called heterocysts (30).

Cyanobacterial mutants impaired in cell division were identified after chemical mutagenesis (21–26, 51). Filamentous mutants were either septate (and thus impaired in cell separation) or serpentine, dividing sporadically to produce long, multinucleoidal cells (24). The gene mutated by random cassette mutagenesis (7, 31) in a septate mutant of *Synechococcus* sp. strain PCC 7942 (13) was characterized; it may be involved in lipopolysaccharide assembly.

Transposon mutagenesis of *Synechococcus* sp. strain PCC 7942. Mutagenesis of PCC 7942 with transposon Tn*901* was used to identify a methionine-biosynthetic gene and genes involved in nitrate assimilation (35, 36, 47). The utility of Tn*901* is limited by its low frequency of transposition (18). Use of Tn*5* in PCC 7120 (4) was enhanced by variant Tn*5*-1058 and derivatives that had (i) stronger expression of the antibiotic resistance operon, (ii) enhanced transposition, and (iii) an internal origin of replication that facilitates recovery of mutated genes (for examples, see references 3, 8, 16, and 49). Tn*5* derivative Tn*5*-692 (in plasmid pRL692; GenBank accession no. AF424805) confers resistance to erythromycin, spectinomycin, and streptomycin; contains a pMB1 *oriV*; and bears mutations (52) that increase its rate of transposition ca. 100-fold relative to that of Tn*5*-1058 (49), providing large numbers of transposon mutants of *Anabaena variabilis* strain ATCC 29413 (PCC 7937) (our unpublished observations) and of PCC 7942.

Wild-type PCC 7942 and its derivatives were grown in BG11 medium (42), and wild-type PCC 7120 and its derivatives were grown as described by Hu et al. (20) in 125-ml Erlenmeyer flasks at 30°C in the light (ca. 3,500 ergs cm^{-2} s^{-1}) on a rotary shaker. Antibiotics were added as appropriate. *E. coli* was grown and transformed as described previously (44). Tn*5*-692 was transferred to PCC 7942 and PCC 7937 by conjugation with *E. coli* strain HB101 bearing pRL443, pRL528, and pRL692 (10, 14). Filters bearing exconjugants were incubated for 48 h at 30°C (light intensity, 1,500 ergs cm^{-1} s^{-1}) before transfer to medium containing erythromycin and spectinomycin (10 μg of each ml^{-1}). Colonies appeared 10 to 15 days later. The frequency of transposition was ca. 3×10^{-5} to 6×10^{-5} per recipient cell. Extensively spreading, filamentous mutants appeared at a frequency of ca. 6×10^{-7} per recipient cell.

The cells of two such spreading mutants, FTN2 (Fig. 1C) and FTN6 (Fig. 1B), are up to 100-fold and 20-fold longer than wild-type cells, respectively (Fig. 1A). The growth rate of these mutants in liquid medium appeared to differ little from that of the wild type. Because their septation was not easily visualized by light microscopy, the cells were negatively stained with uranyl acetate and examined by electron microscopy. Sites of cell division in mutants FTN2 (Fig. 2B and C) and FTN6 (Fig. 2D and E) are much less frequent than in the wild-type strain (Fig. 2A). Spreading of the mutant colonies may be a consequence of the lengths of the individual cells providing a larger step size in a random-walk process of colonial growth. We use the designation "FTN" to suggest filamentation.

To clone *oriV*-containing Tn*5*-692, which lacks sites for *Sal*I and *Bln*I, together with DNA contiguous with it, DNA recovered from FTN2 was cut separately with *Sal*I and *Bln*I, circularized with T4 DNA ligase, and transformed to *E. coli* DH5α, yielding plasmids pRL2462 and pRL2733, respectively. DNA recovered from FTN6 was cut with *Sal*I and similarly treated, yielding pRL2463. PCC 7942 was transformed with pRL2462 and pRL2463 (29). All spectinomycin- and erythromycin-resistant transformants were filamentous, establishing that the mutations were closely linked to the transposon. Mutants FTN2 and FTN6 were completely segregated, and the transposon is

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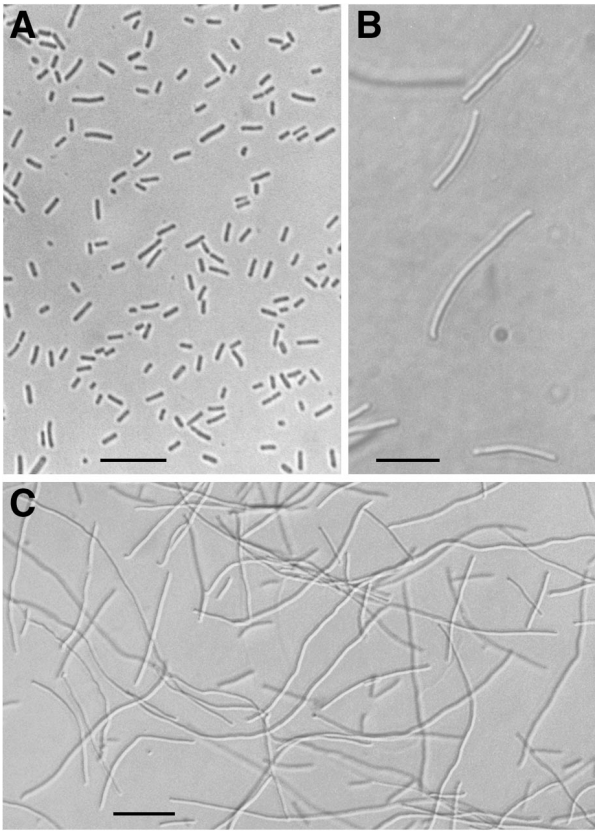

FIG. 1. Morphology of wild-type PCC 7942 (A) and of mutants FTN2 (C) and FTN6 (B), grown in liquid medium and visualized by bright-field light microscopy. Scale bars represent 12.5 μm (A and B) or 25.6 μm (C).

present in single-copy open reading frames (ORFs; data not shown) that we have provisionally designated *ftn2* and *ftn6*.

DNA contiguous with the transposon was subcloned from pRL2462 to pBluescript SK(+) (Stratagene, La Jolla, Calif.) as *Xba*I-*Sal*I and *Spe*I-*Sal*I fragments, producing plasmids pRL2466 and pRL2468, respectively, and from pRL2463 to pBluescript SK(+) as *Xba*I-*Sal*I and *Spe*I-*Spe*I fragments, producing plasmids pRL2465 and pRL2464, respectively. Part of plasmid pRL2733 was sequenced with primers. The expected 9-bp duplication adjacent to the site of insertion of the transposon was found in the case of FTN6 but, curiously, the two transposon-proximal 9-bp sequences recovered from FTN2 differed at one position [TGCAGGCG(C/T)]. To compare the sequences determined with the transposon-mutated genes with those from the wild-type sequences, genomic DNA from wild-type PCC 7942 was isolated (29) and the two wild-type genes were amplified piecewise by PCR and sequenced. Independent PCR amplifications confirmed the sequence TGCAGGCGC adjacent to the transposon in FTN2. Except for the final 183 bp of *ftn2*, which were sequenced only from pRL2733 as template, all portions of *ftn2* and *ftn6* were sequenced on both strands of DNA derived from a transposon recovery and on both strands of DNA PCR amplified from PCC 7942. Where there was any possible inconsistency, multiple independent PCR products were sequenced. Our sequence data include the final 282 bp of an ORF 3′ from *ftn2* and the first 651 bp of an ORF 3′ from *ftn6*. Corresponding 3′ ORFs appear in sequence data from *Synechococcus* sp. strain PCC 6301 that were kindly provided by M. Sugita.

ftn2 and its downstream neighboring ORF are oppositely oriented and separated by a possible transcriptional terminator (CGCAaGGGGTgaaCCCCcTGCG [lowercase letters show deviations from the palindrome]), whereas *ftn6* and its downstream ORF are carried on the same strand of DNA. Therefore, the phenotype of FTN2 is not, although the phenotype of FTN6 may be, attributable to a polar effect of the mutation on a downstream gene. Whereas the 152-amino-acid (aa) Ftn6 shows significant similarity (Expect value [*E*] [an Expect value is a BLAST indicator expressing the statistical significance of the alignment found and indicates the number of times one might expect to see such a match merely by chance] = 10^{-8} to 3×10^{-6}) only to predicted proteins from other cyanobacteria, the predicted product of the parallel downstream ORF shows greater similarity to endopeptidase A of *Arabidopsis* (BLAST score, 616; $E = 10^{-175}$) and of other cyanobacteria (BLAST scores, 684 to 653; $E < 10^{-180}$) than to endopeptidases A from other bacteria ($E \geq 10^{-116}$).

ftn2 predicts a 631-aa protein, Ftn2, that shows greatest similarity to the predicted products of *Anabaena* sp. strain PCC 7120 ORF *all2707* (28), which we have designated $ftn2_A$ (BLAST score, 278; $E = 3 \times 10^{-75}$ [1]), a *Nostoc punctiforme* ORF (BLAST score, 263; $E = 10^{-70}$), and presumptive gene *sll0169* of *Synechocystis* sp. strain PCC 6803 (BLAST score, 218; $E = 2 \times 10^{-55}$). Ftn2 also shares similarity with an *Arabidopsis* protein (AB016888; $E = 10^{-11}$). The InterProScan program (http://www.ebi.ac.uk/interpro/scan.html) shows that Ftn2 and its cyanobacterial and plant orthologs have a DnaJ N-terminal domain (aa 6 to 70). Otherwise, they are dissimilar from other division-related proteins (6), as is the case with

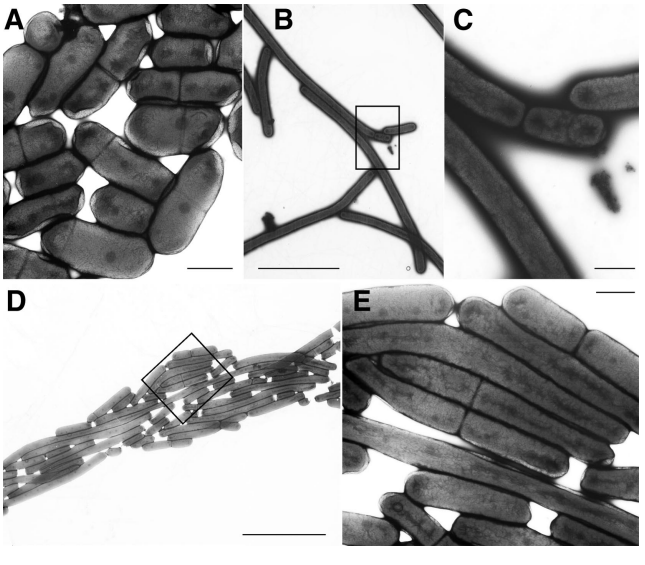

FIG. 2. Structures of wild-type PCC 7942 (A) and of mutants FTN2 (C; see box in panel B) and FTN6 (E; see box in panel D), negatively stained with uranyl acetate and examined by electron microscopy. The cells of both mutants divide infrequently. Scale bars represent 1 μm (A, C, and E) or 10 μm (B and D).

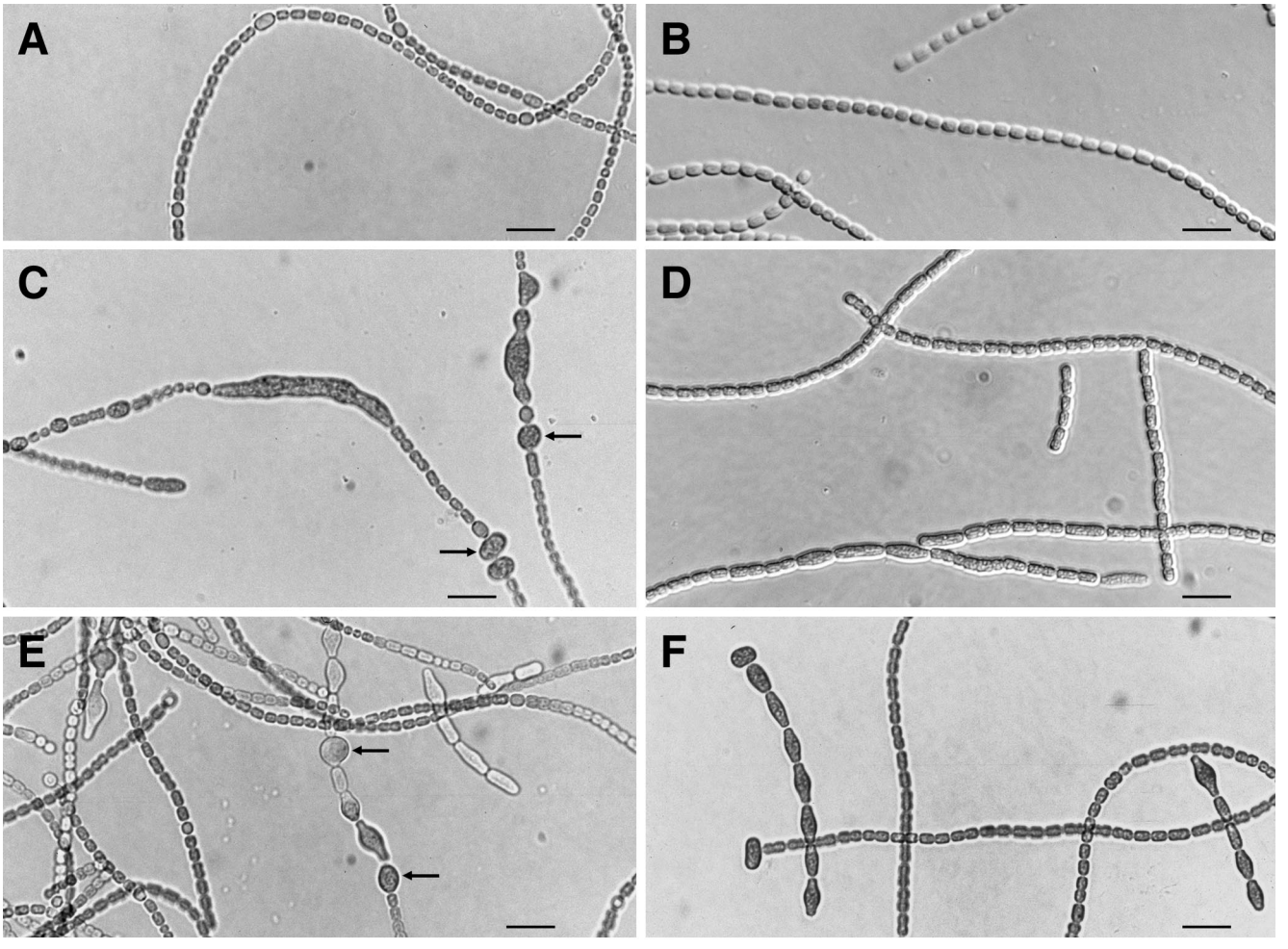

FIG. 3. Morphology of *Anabaena* sp. strain PCC 7120 wild type (A and B) and mutants $FTN2_A$ (C and D) and $FTN6_A$ (E and F) grown in liquid medium free of combined nitrogen (AA/8 [20]) (A, C, and E) or supplemented with 5 mM nitrate (B, D, and F). Scale bars represent 12.5 μm. Akinete-like cells are indicated by arrows.

FTN6 and its orthologs. The presence in Ftn2 of a DnaJ domain, a single tetratricopeptide repeat (TPR) (aa 136 to 169; shown by the same program), and a leucine zipper pattern (aa 234 to 255, Prosite protein; PROSITE program at [http://ca.expasy.org/tools/scanprosite/]) suggests that Ftn2 may function as part of a complex with one or more other proteins and may be regulatory.

Proteins of the DnaJ-domain family are chaperonins that have a highly conserved J domain of approximately 70 aa, often found near the N terminus, that mediates interaction with DnaK and regulates the ATPase activity of the latter (9). *dnaJ* shares with *fts* genes the property that its inactivation leads to a filamentous phenotype (41). The *dnaJ* gene of *Synechococcus* sp. is required for growth (39).

The TPR, typically 34 aa in length and first described for the yeast cell division cycle regulator Cdc23p (46), was later found in many other proteins (11, 17, 32). Although frequently present in tandem arrays of 3 to 16 copies, single (as in FTN2) or paired TPRs are also common (32). Diverse processes involve TPR proteins, including cell-cycle control (32). No biochemical commonality connects TPR-containing proteins, although the TPR forms scaffolds that mediate protein-protein interactions and, often, the assembly of multiprotein complexes. The *Arabidopsis* Ftn2 ortholog is predicted to have a chloroplast transit peptide (http://HypothesisCreator.net/iPSORT/) and so may play a role in chloroplast division.

Inactivation of the *ftn*$_A$ genes of *Anabaena* sp. strain PCC 7120. Orthologs *ftn2*$_A$ (see above) of *ftn2* and *ftn6*$_A$ (*all1616*) (BLAST score, 76; $E = 10^{-13}$) of *ftn6* were identified in the genome of PCC 7120 (http://www.kazusa.or.jp/cyano/Anabaena). *ftn2*$_A$ is transcribed on the same strand of DNA as its downstream gene, whereas *ftn6*$_A$ is oriented opposite to its downstream gene. To inactivate *ftn2*$_A$ and *ftn6*$_A$, a copy of each, truncated at both ends, was prepared by PCR with PCC 7120 DNA as template and primer pair 5′-CCGAATTCGTGGCAGTGGAAAATCGTGGG-3′ and 5′-CCGAATTCCACTTGCACGATTGGGATC-3′ and primer pair 5′-CCGAATTCGCCCTACTCATTAACTATAG-3′ and 5′-CCGAATTCCGGAGCGATCGCTTGTTTG-3′, respectively. The copies were cloned in the *Eco*RI site of pRL498 (15), producing plasmids pRL2471 and pRL2474, respectively. The clones were transferred by conjugation (14) to wild-type PCC 7120 with selection on AA

plus nitrate agar medium containing 25 μg of neomycin ml^{-1}. Homologous recombination was confirmed by Southern blotting (data not shown).

Anabaena sp. strain PCC 7120, a filamentous cyanobacterium, is capable of cellular differentiation (48). Cells of *ftn2*$_A$ (Fig. 3D) and *ftn6*$_A$ (Fig. 3F) *Anabaena* sp. strains, i.e., of PCC 7120::pRL2471 and PCC 7120::pRL2474, grown in the presence of nitrate were often up to twice as long as cells of the wild-type strain (Fig. 3B). In medium free of combined nitrogen, *ftn2*$_A$ (Fig. 3C) and *ftn6*$_A$ (Fig. 3E) mutants formed very elongated vegetative cells (those of *ftn2*$_A$ were up to 60-fold longer than those of the wild-type strain [Fig. 3A]), heterocysts of nearly normal size (but also sometimes up to fourfold larger, with an increase in both length and width), and enlarged akinete-like cells. Unlike mutant FTN6$_A$, mutant FTN2$_A$ failed to segregate completely (data not shown). Wild-type PCC 7120 bears many copies of insertion sequences, including those in several ORFs whose putative products resemble protein kinases (40). Therefore, PCC 7120, which does not normally form akinetes in the laboratory, may itself exhibit a mutant phenotype. The presence of the greatly enlarged cells, which by their shape and frequent contiguity to heterocysts somewhat resemble akinetes, suggests that Ftn2$_A$ and Ftn6$_A$ may be involved in cellular differentiation as well as in division. The functions of *ftn2*, *ftn2*$_A$, *ftn6*, and *ftn6*$_A$ remain to be elucidated.

Nucleotide sequence accession number. The sequences of *ftn2* and *ftn6* have been submitted to GenBank under accession no. AF421196 and AF421197.

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